Appl Biochem Biotechnol (2009) 153:84–93
DOI 10.1007/s12010-008-8448-0

Wheat Straw Autohydrolysis: Process Optimization and Products Characterization

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Received: 23 May 2008 / Accepted: 18 November 2008 /
Published online: 10 December 2008

Abstract Wheat straw was subjected to autohydrolysis treatments in order to selectively hydrolyze the hemicellulose fraction. The effects of temperature (150–240°C) and non-isothermal reaction time on the composition of both liquid and solid phases were evaluated and interpreted using the severity factor (log R_0). The operational conditions leading to the maximum recovery of hemicellulose-derived sugars were established for log R_0=3.96 and correspond to 64% of the original (arabino)xylan with 80% of sugars as xylooligosaccharides. Under these conditions, a solubilization of 58% xylan, 83% arabinan, and 98% acetyl groups occurred. Glucan was mainly retained in the solid phase (maximum solubilization 16%), which enables an enrichment of the solid phase to contain up to 61% glucan. Delignification was not extensive, being utmost 15%. The yields of soluble products, including sugars, acetic acid, and degradation compounds, such as, furfural, 5-hydroxymethylfurfural obtained suggest the fitness of liquid stream for fermentation purposes or to obtain xylooligosaccharides with potential applications in food, pharmaceutical, and cosmetic industries.

Keywords Autohydrolysis · Pre-treatment · Wheat straw · Xylooligosaccharides

Introduction

Wheat straw is an agricultural residue that presents many interesting characteristics that facilitate its biotechnological upgrade in a biorefinery framework [1], namely, it is an herbaceous crop, soft material that can be transported in relatively high density form and typically has a low water content that enables its easy storage. It is a very abundant material and it does not present an excessive commercial value. Currently, it is used for low value applications such as mulch [2], animal-feed and bedding [3, 4] and also for energy [5] and

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pulp production [6]. Actually, it is considered to be the crop residue that presents the highest potential for the production of second generation bioethanol in Europe, and it is only surpassed by corn stover and rice straw in North America and Asia, respectively [7].

As for corn stover and rice straw, the main constrains for the upgrading of this material are related to its macromolecular composition. Although it has high polysaccharide content it also has a significant pentose content that can account up to 30% of the feedstock [8] that still imposes some restrictions on polysaccharide bioconversion to ethanol. Therefore, an integrated biorefinery approach that enables the selective fractionation of wheat straw into its main macromolecular components and its subsequent individual upgrade may be advantageous.

On the process options for the selective fractionation of the lignocellulosic components are dilute acid hydrolysis [9, 10], alkali treatments [11], and hydrothermal treatments such as steam-explosion [12, 13], wet oxidation [14], microwave treatment [15], and autohydrolysis [8, 16]. Among these, autohydrolysis has the advantage to enable a high recovery of hemicelluloses as soluble saccharides, while both cellulose and lignin could be recovered in the solid phase with minor losses. Furthermore, it has many technological and environmental benefits, mainly related to its uncatalyzed nature. Specifically, comparing to acid hydrolysis, autohydrolysis induces a lower byproduct generation, limited equipment corrosion problems, and reduction of operational costs since further neutralization can be omitted.

The solid fraction obtained from the autohydrolysis treatments can be used for the production of bioethanol or some biobased added-value products [17, 18]. From the liquid hemicellulose-rich stream, it is possible to extract phenolic antioxidants [19] and apart from ethanol by fermentation, there are also some added-value products that can be produced. Examples include xylooligosaccharides (XOS) that can be directly obtained by autohydrolysis [20] or xylitol, after bioconversion [21, 22], which can have applications in food and pharmaceutical industries. Due to advantageous technological properties, i.e., high thermal stability in a wide range of pH (2.3–8) and long shelf life, together with their bifidogenic effect, XOS can be used as food ingredients, although pharmaceutics and cosmetic applications also hold a promise of high potential for this compounds [16, 23].

In this work, wheat straw was subjected to autohydrolysis treatments in order to selectively hydrolyze the hemicellulose fraction. The effects of temperature and non-isothermal reaction time on the composition of both liquid and solid phases were evaluated and interpreted using the severity factor (log R_0).

Methods

Feedstock Material

Wheat straw was supplied by Estação Nacional de Melhoramento de Plantas (Elvas, Portugal). The feedstock material was ground with a knife mill to particles smaller than 1.5 mm, homogenized in a defined lot, and stored in plastic containers at room temperature. The dry matter content was 92%.

Autohydrolysis of Wheat Straw

The hydrothermal treatments (autohydrolysis) were performed in a stainless steel reactor (Parr Instruments Company, Moline, Illinois, USA) with a total volume of 600 mL. The

reactor was fitted with two four-blade turbine impellers, heated by an external fabric mantle, and cooled by cold water circulating through an internal stainless steel loop. Temperature was controlled through a Parr PID controller, model 4842. The wheat straw was mixed with water in the reactor in order to obtain a liquid to solid ratio (LSR) of 10 (kg water/kg dry feedstock). The agitation speed was set at 150 rpm and the reactor heated to reach final temperatures ranging between 150°C and 240°C (non-isothermal conditions). Typically, the average heating rate (from 100°C) was 3.8°C/min. When the desired temperature was attained, the reactor was rapidly cooled down and the liquid and solid phases were recovered by filtration (Whatman filter paper no. 1).

The effects of time and temperature on wheat straw autohydrolysis are interpreted based on the severity factor, log R_0 [24]:

$$R_0 = \int_0^t \exp\left(\frac{T(t) - 100}{14.75}\right) dt$$

where t is time (min), T the temperature (°C), and 14.75 an empirical parameter related with activation energy and temperature. The solid phase was washed with water at room temperature, dried at 40°C and the yield and composition were determined as described below.

Analytical Methods

Chemical Characterization of Feedstock and Processed Solids

The materials were ground in a knife mill to a particle size <0.5 mm and the moisture content was determined by oven-drying at 105°C to constant weight. The ash content was determined by igniting the contents at 550°C for 5 h. The samples were analyzed for glucan, xylan, arabinan, and acetyl groups after quantitative acid hydrolysis with 72% (*w*/*w*) H_2SO_4 (60 min, 30°C) followed by hydrolysis with 4% (*w*/*w*) H_2SO_4 (60 min, 121°C). The acid insoluble residue was considered as Klason lignin, after correction for ash. The monosaccharides and acetic acid in the hydrolyzates were analyzed by HPLC as described below. Protein quantification was performed by the Kjeldahl method using the N×6.25 conversion factor.

Chemical Characterization of Liquors and Hydrolyzates

Glucose, xylose, arabinose, acetic acid, 5-hydroxymethylfurfural (HMF), and furfural were analyzed by HPLC (Waters, Milford, USA) using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) as previously described [20]. All samples were filtered through 0.45 μm membranes before analysis. A sample of the liquors was directly analyzed by HPLC. Another sample was mixed with 72% (*w*/*w*) H_2SO_4 in order to obtain 4% (*w*/*w*) H_2SO_4 final concentration in the reaction medium and hydrolyzed at 121°C for 60 min to convert soluble hemicellulose into their constituent sugar monomers. Oligosaccharides concentrations were expressed as the increase in sugar monomers, as analyzed by HPLC, after liquor hydrolysis. For calculation purposes, xylooligosaccharides (XOS) were considered as arabinose substituted xylooligosaccharides.

Total phenolic compounds content was assayed spectrophotometrically by the modified Prussian blue method [25] using tannic acid as calibration standard.

All the reported results are the average of at least two replicates (typical analytical error <5%).

Results and Discussion

Feedstock Composition

Chemical composition of wheat straw may vary depending on the wheat variety and culture conditions. Table 1 shows the composition of the wheat straw used in this work. It contains 60% (*w*/*w*) of total polysaccharides, from which approximately 2/3 is cellulose, as estimated from the glucan content. This value favorably compares with previously reported values [9, 16], presenting higher cellulose content. Wheat straw hemicellulose corresponds to a β-D-(1,4)-linked xylopyranosyl backbone, substituted with arabinofuranose, 4-*O*-methylglucuronic acid, acetyl groups, and xylose as well as phenolic acids [26]. The content of hemicellulose, and specifically the amounts of xylose, arabinose, and acetyl groups, are also similar, to values previously reported [9, 16]. Concerning the insoluble lignin content some differences occur. The value found is only slightly higher than the reported by Nabarlatz et al. [16] but lower than the obtained by other authors [9, 22]. Furthermore, due to the presence of ash, Klason lignin analysis may give an overestimation of the lignin content. Therefore, in this work, the values obtained were corrected for the ash content of acid insoluble residue (10.2%), which partially explains the differences found.

Effect of Autohydrolysis on the Products Yields

Hydrolysis Profile

Figure 1 shows the variation in xylan, arabinan, glucan, oligosaccharides, monosaccharides, furan derivatives, and Klason lignin recovery as a function of the severity factor. As expected, autohydrolysis mainly affected hemicellulose components (Fig. 1A). The hydrolysis of xylan is negligible up to a severity factor of 3.25, from which it decreases sharply to reach 97% solubilization of the original xylan at the severest condition assayed. The highest recovery of xylooligosaccharides (XOS), 10.5 g/100 g feedstock, was obtained for the severity value of 3.96 and corresponds to 50% of feedstock (arabino)xylan. In these conditions, 58% and 83% of the original xylan and arabinan, respectively, were solubilized, and 64% of the original (arabino)xylan was recovered as soluble saccharides. The XOS yield obtained is higher than the 43% previously reported for the autohydrolysis of the same feedstock and very similar to those described for almond shells [16], although higher

Table 1 Average macromolecular composition of wheat straw (% of dry weight).

Component	This work[a]	Kabel et al. [9]	Nabarlatz et al. [16]
Cellulose[b]	38.9±0.2	31	28.4±4.2
Hemicellulose	23.5	24.2	22.5
Xylan	18.1±0.3	20	17.4±2.8
Arabinan	3.0±0.2	2.5	2.5±0.3
Acetyl groups	2.5±0.1	1.7	2.6±0.9
Klason lignin	18.0±0.5	25	15.9±0.3
Ash	9.70±0.03	–	6.39±0.04
Protein	4.5±0.5	–	–
Others (by difference)	5.5	–	14.7

[a] Average of three replicates

[b] Measured as glucan

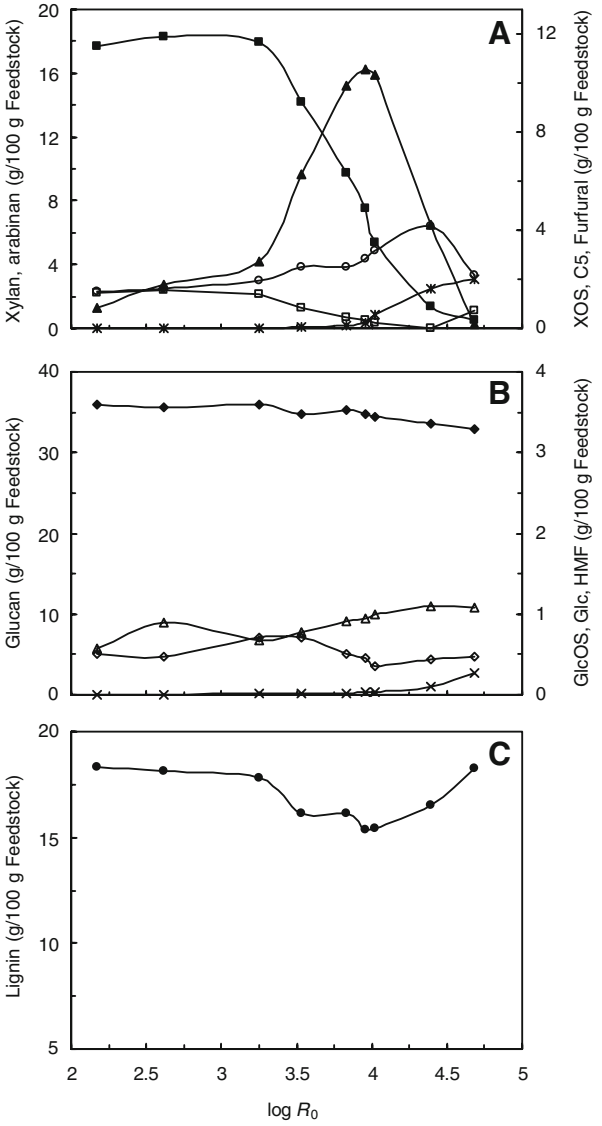

Fig. 1 Xylan, arabinan, glucan, and derived products and Klason lignin as a function of severity. (Xylan *filled square*, XOS *filled upright triangle*, C5 (Xyl+Ara) *empty circle*, Arabinan *empty square*, Furfural *asterisk*, Glucan *filled diamond*, *GlcOS empty diamond*, Glc *empty upright triangle*, HMF *x symbol*, Klason lignin *filled circle*)

values have been obtained using brewery spent grains (BSG) or corn cobs [20, 27]. Monomeric pentoses yield increased up to a severity factor of 4.36, reaching a value of 4.2 g/100 g feedstock. A further increase in the severity led to a decrease in the recovery of these monosaccharides, owing to degradation reactions. In fact, the degradation into furfural becomes measurable from the severity factor of 3.25, and increased to reach 2.0 g/100 g feedstock for the harsher conditions assayed (Fig. 1A).

In contrast to hemicellulose components, glucan essentially remained in the solid phase and only a small part of it was depolymerized to oligosaccharides and glucose (Fig. 1B). The maximum degradation of glucan occurred at the severest condition with 16% of the original glucan being solubilized. The maximum yield of glucose, 1.10 g/100 g feedstock, was obtained in those conditions and corresponds to 2.6% of feedstock glucan. The

maximum yield of gluco-oligosaccharides (GlcOS) was even lower than the obtained for glucose (0.71 g/100 g feedstock) and was obtained for a lower severity (log R_0=3.25). In all conditions, HMF concentrations were also low, reaching at the maximum a value of 0.28 g/100 g feedstock.

The effect of autohydrolysis in Klason lignin recovery is shown in Fig. 1C. Up to a severity of log R_0=3.25 almost no solubilization of lignin occurred. Higher severities led to an increase in lignin degradation being the highest value attained for a severity of log R_0= 3.96. In these conditions, 14.6% of the original lignin was solubilized. A further increase in severity, led to an increase in Klason lignin recovery. This increase is quite typical for the hydrothermal processes and was already described both for the autohydrolysis and dilute acid hydrolysis of BSG [20, 28] and can be associated to condensation reactions of lignin with sugar and/or sugar degradation products to give insoluble products (pseudo-lignin) [29–31].

Effect of Autohydrolysis on the Composition of the Liquid Phase

Table 2 shows the composition of the liquors obtained for different severity conditions. Xylooligosaccharides (XOS) were the main components of liquors for almost all conditions assayed, being xylose the main monosaccharide obtained. The concentration of XOS increased with severity to reach a maximum at log R_0=3.96. The oligosaccharides are almost devoid of acetyl groups and the substitution degree with arabinose is also low and decreased with the increase of severity up to log R_0=4.02. In the conditions leading to the highest recovery of XOS, these oligosaccharides contained eight arabinose and three acetyl substituents, respectively, per each 100 xylose units which compares to the initial substitution of xylan chain (17 arabinose and 31 acetyl groups per 100 xylose units) indicating that an efficient and selective removal of the substituents. The results obtained

Table 2 Composition (g/L) of the liquors obtained from autohydrolysis of wheat straw.

	Severity factor, log R_0								
	2.17 (150)	2.62 (170)	3.25 (190)	3.53 (200)	3.83 (210)	3.96 (215)	4.02 (220)	4.39 (230)	4.68 (240)
pH	6.28	5.61	5.11	4.83	4.32	4.28	4.25	3.87	3.70
XOS	0.81	1.75	2.69	6.09	9.54	10.1	9.87	4.05	0.17
GlcOS	0.50	0.47	0.70	0.69	0.48	0.44	0.34	0.42	0.45
AcO	n.a.	0.05	n.a.	0.03	0.21	0.14	0.40	n.a.	n.a.
Xyl/Ara[a]	0.6	0.9	2.8	5.1	9.3	13.4	19.9	8.2	1.3
Xylose	1.66	1.79	1.44	1.63	1.62	1.88	2.23	3.77	2.34
Arabinose	n.d.	n.d.	0.70	1.15	1.16	1.19	1.19	0.78	n.d.
Glucose	0.63	0.97	0.73	0.84	0.98	1.01	1.06	1.17	1.15
Acetic acid	0.64	0.80	1.31	1.70	2.06	2.72	2.77	2.96	3.19
HMF	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.08	0.20
Furfural	n.d.	n.d.	0.01	0.03	0.08	0.16	0.38	1.14	1.37

Values in parentheses indicate the reaction temperature

XOS arabinose substituted xylooligosaccharides, *GlcOS* gluco-oligosaccharides, *AcO* acetyl groups linked to oligosaccharides, *n.d.* not detected, *n.a.* not available

[a] Ratio xylose/arabinose in XOS (mol/mol)

are also in agreement with the previous reported by Liavoga et al. [32] for wheat straw XOS obtained by autohydrolysis that also contained a similar level of arabinose substitution.

The low content of acetyl groups of these oligosaccharides contrasts with the results obtained for other materials also using autohydrolysis [27, 33, 33]. This can be partially explained by the lower acetyl groups content of wheat straw, although this did not occur for the BSG OS, obtained from a feedstock with lower acetyl groups content [20].

Acetic acid concentration increased steadily with severity and do not significantly increase after a post-hydrolysis, which is consistent to low substitution of oligosaccharides with acetyl groups. A similar trend was found for the soluble phenolic compounds (data not shown).

Concerning the composition of sugar degradation products such as furfural and HMF, the concentrations obtained were very similar to those reported for the autohydrolysis of eucalypt but lower than those described for the autohydrolysis of BSG and corn cobs [20, 27]. In fact, the generation of sugar degradation products was quite low, which is an advantage that enables the utilization of autohydrolysis to produce XOS-rich liquors that can be used both to obtain purified XOS mixtures (which can be potential prebiotic ingredients) or for the production of pentoses-rich culture media, e.g., using a enzymatic hydrolysis or a dilute acid post-hydrolysis as specifically optimized for wheat straw oligosaccharides [34].

The levels of fermentation-inhibiting compounds are very low suggesting the fitness of hydrolyzates for fermentation purposes as already demonstrated [21].

Effect of Autohydrolysis on the Composition of the Solid Phase

Figure 2 shows the solid residue yield and composition of the processed solids as a function of hydrolysis severity. For the less severe conditions, the solid solubilization that occurred is very low and polysaccharides and lignin content is very similar to the feedstock (Table 1). However, from the severity factor of log R_0=3.25 the solid yield decreases sharply to reach about 55%. This decrease could be correlated to the solubilization of hemicellulose components (r=0.95, data not shown). Within hemicellulose, acetyl groups are the first to be hydrolyzed, followed by arabinan and xylan. Total removal of acetyl groups and arabinan from the solid phase was obtained for less severe conditions (log R_0=4.02 and 4.39, respectively) than for xylan. These results are in good agreement with previous

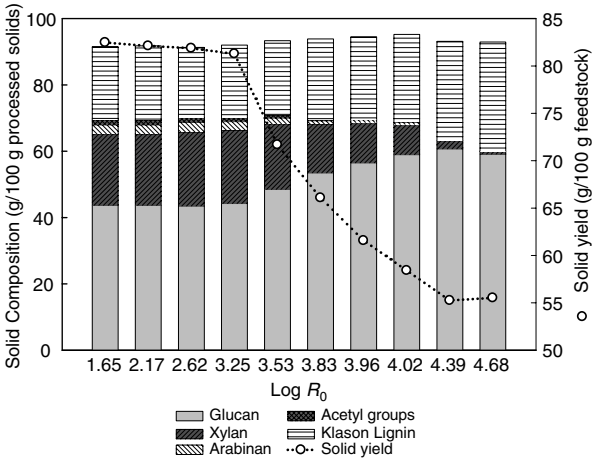

Fig. 2 Effect of the severity factor on the solid yield and composition of processed solids obtained after autohydrolysis of wheat straw

reports for other raw materials. Garrote et al. [33] also observed a similar kinetic trend to these components for eucalypt wood. Comparing to other pre-treatment options for wheat straw, the hydrothermal processing performed in this work enabled a higher xylan solubilization than dilute sulfuric acid pre-treatment or steam-explosion followed by alkaline peroxide post-treatment [26, 35].

Glucan was almost not affected by the hydrolytic treatment and a solid residue with increased glucan content was obtained. These results are in good agreement with the previous obtained for wheat straw [32] and for other similar feedstocks, e.g., barley straw and rice husks [16]. In the case of wood, little cellulose degradation used to occur at temperatures below 230°C [30]. The Klason lignin content follows a similar pattern to glucan, except that the maximum solubilization of lignin (15%) occurred for a severity factor of 3.96 (corresponding to the maximum hemicellulose-derived sugars yield) whereas the highest glucan solubilization was achieved for the severest condition assayed. However, the solubilization of lignin is relatively low demonstrating that hydrothermal treatment does not significantly interact with lignin. In fact, the values obtained are even lower than the reported for the autohydrolysis of corn cobs under similar operational conditions [36] and also lower than other reported pre-treatments, specially those including either alkaline or oxidative treatments [37, 38].

Since hemicellulose removal disrupts the material structure possibly increasing porosity, it is expected that the solid residue produced may have an improved enzymatic digestibility [39]. In fact, xylan solubilization is generally considered to be the main mode of action to improve enzymatic hydrolysis although in some cases lignin disruption/removal can also be relevant for further enzymatic hydrolysis [40–42]. Although delignification was not carried out in this work, it will be pursued if the future results on enzymatic hydrolysis tests indicate that it will be important. However, conversely to other chemical pre-treatments, autohydrolysis has the advantage, even with some physical disruption and chemical solubilization, to render lignins that retain much of its native chemical composition that can be upgraded in a specific lignin upgrade stream.

Conclusions

Autohydrolysis enabled the selective solubilization of wheat straw hemicellulose with a high recovery of XOS, under relatively mild conditions. The optimum conditions were found for 215°C (log R_0=3.96). Under these conditions, an important glucan enrichment of the solid phase, together with lignin, was possible.

Thus, it can be stated that autohydrolysis is an appropriated pre-treatment to upgrade lignocellulosic materials leading to valuable co-upgrade solutions in a biorefinery framework.

Acknowledgements Authors are grateful to Fundação para a Ciência e a Tecnologia (FCT) for the financial support of this work (project BIOREFINO PTDC/AGR-AAM/71533/2006). Talita Silva-Fernandes gratefully acknowledges the grant funded by CEBio (Prime-IDEIA-AdI Project no. 70/00326).

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